Filamentous Capsulated Streptococci from the Human Respiratory Tract

III. Immunochemical Studies of the Cross-Reactivity Between the Cell Wall Antigens of a Filamentous Streptococcus and of Pneumococcus

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A cell wall-associated polysaccharide has been isolated from a noncapsulated mutant, 89R50, of the alpha-hemolytic filamentous streptococcus of provisional capsular type 89. Chemical analysis of the polysaccharide indicates that it consists of galactosamine, glucosamine, glucose, and phosphorus. Immunological cross-reactivity was observed between the streptococcal cell wall antigen and antibody to the cell wall-like capsular polysaccharide of pneumococcus. This cross-reactivity appears attributable to similarities in the teichoic acid moieties rather than in the mucopeptide moieties of the two polymers. No chemical or immunological differences were observed in the cell wall carbohydrate of the noncapsulated streptococcus, 89R50, and that of its capsulated progenitor. It is suggested that antibody to this cell wall polysaccharide might serve as a basis for grouping other filamentous strains of streptococci of diverse capsular types, the cell wall antigens of which show similar cross-reactivity with that of pneumococcus.

Previous studies have indicated that immunological cross-reactivity exists between the cellular antigens of some strains of alpha- and nonhemolytic filamentous streptococci of the human respiratory tract and the C, or cell wall, polysaccharide of pneumococcus (2). In an extension of the initial observations, a cell wall carbohydrate, reacting with an antiserum to the C, or cell wall-like capsular polysaccharide of pneumococcus (5) and consisting of glucose, galactose, glucosamine, galactosamine, and phosphorus, was isolated from the prototypic strain of the alpha-hemolytic filamentous streptococcus provisionally designated type 83 (16). Studies of the cell wall antigen of this organism demonstrated that an immunological similarity existed between it and the cell wall antigen of pneumococcus, but the immunochemical basis for this cross reactivity was not defined.

The C and Cα polysaccharides of pneumococcus are composed of two polymeric subunits, a mucopeptide and a teichoic acid (6, 11, 23). The teichoic acid moiety of the C polysaccharide consists of a repeating unit of N-acetylglactosaminyl-glycosyl-N-acetyl-diaminotriideoxy-hexosyl-ribitol-5-phosphate (6), with choline phosphate attached to the ribitol residues of the backbone structure (23). This teichoic acid polymer in turn is linked to the mucopeptide moiety to form the macromolecular structure of pneumococcal C polysaccharide (11).

Immunological analysis of pneumococcal C polysaccharide by Gotschlich and Liu (11) indicated clearly that this polymer had two major antigenic determinants, the teichoic acid and the pentapeptide moiety of the mucopeptide. Schiffman et al. (28) extended these observations by demonstrating that the mucopeptide moiety or the region of its attachment to the teichoic acid might also serve as immunochemical determinants of pneumococcal C and Cα polysaccharides.

The studies described below indicate that the immunological cross-reactivity observed between the cell wall antigen of a filamentous alpha-hemolytic streptococcus and the C polysaccharide of pneumococcus is dependent, at least in part, upon the structural similarities of their teichoic acids and not upon their cell wall mucopolysaccharides.

MATERIALS AND METHODS

Bacterial strains. The prototypic strain of the filamentous alpha-hemolytic streptococcus of provi-
sional capsular type 89 (2), isolated from respiratory secretions of a patient at the Philadelphia General Hospital and referred to hereafter as type 89, was used. A noncapsulated mutant of this strain, design-
nated streptococcus 89R50, was isolated after 50 serial daily passages of type 89 in beef heart infusion-
Neopeptone (Difco Laboratories, Detroit, Mich.) broth (1) containing 20% homologous antcapsular serum. Pneumococcal strain R36NC is a noncaps-
nulated mutant of the type II capsulated strain, D39S (3). The noncapsulated group B streptococcal strain was obtained from Rebecca Lancefield, The Rockefeller University, New York, New York. Strains of Escherichia coli, Bacillus sp. and Clostridium sp. were isolated originally at Barnes Hospital, St. Louis, Mo.

Preparation of cell walls, group-specific carbo-
hydrates and mucopolysaccharides. Cell walls and muco-
peptides were prepared by methods described previ-
ously (4, 13, 14). Group-specific carbohydrates were ex-
tracted from the cell walls of streptococcal strain 89R50 and from pneumococcal strain R36NC with cold trichloroacetic acid according to the method of Park and Hancock (24).

Analytical methods. Methods for the assay of glucose, glucosamine, galactosamine, muramic acid, rhamnose, and amino acids were those described previously (8, 9, 17, 26, 30). Total phosphorus was measured by the method of Chen et al. (7).

Ion-exchange chromatography. Diethylamino-
ethyl-cellulose chromatography was performed by the method described by Karakawa and Krause (17). Paper chromatography was carried out by the method of Pazur et al. (25), using a solvent of butyl alcohol-pyridine-water in a ratio of 6:4:3.

Precipitin analyses. Quantitative precipitin tests were performed by a modification of the method of McCarty and Lancefield (22) described by Karakawa et al. (18). Double diffusion tests in agar gels were done by the method described by Karakawa and Kane (15). Mucoprotease, solubilized by ultrasonic treat-
ment for 15 min in a 20-kc sonic oscillator (14), was used in the precipitin tests.

Protein determinations. Total protein in antibo-
dies to mucoprotein and to carbohydrate was determined by the biuret method (10).

Preparation of antiserum. Antisera were prepared in rabbits by intravenous immunization with forma-
lized, heat-killed vaccines according to a method described previously (3).

RESULTS

Earlier studies have drawn attention to the immunological cross-reactivity between the C₄ capsular polysaccharide of pneumococcus (5) and a variety of strains of capsulated filamentous alpha- and nonhemolytic streptococci of the human respiratory tract (2, 16). Such an immunological relationship is consistent with the observation that the cell walls of pneumo-
coccus and of a filamentous streptococcus stud-
ied previously have in common certain chemical constituents, namely galactosamine, glucosa-
mine, glucose, phosphate, and several amino acids (16). In the following experiments, the immunochemical basis for the cross-reactivity between the cell walls of pneumococcus and of a filamentous alpha-hemolytic streptococcus has been investigated in greater detail.

Isolation of cell wall carbohydrate from the noncapsulated filamentous streptococcal strain 89R50. A culture of the noncapsulated filamentous streptococcal strain 89R50 was grown in 20 liters of Todd-Hewitt broth for 18 h at 37 C. The cells were harvested by centrifuga-
tion, and the packed cells were extracted with 10% cold trichloroacetic acid by the method of Park and Hancock (24). Analysis of the crude trichloroacetic acid extract by double diffusion in agar clearly indicated that an antigen of streptococcus 89R50 shared com-
mon immunological features with the C₄ polysaccharide of pneumococcus. The precipitin line formed by the streptococcal antigen and pneumococcal C₄ antiserum spurred the precipitin line formed by a purified preparation of pneumococcal C polysaccharide and the same antiserum. These results suggested that the cell wall antigen of streptococcal strain 89R50 and the C polysaccharide of pneumo-
coccus possess similar but not identical antigenic determinants.

Table 1 shows a comparison of the chemical composition of the cell wall antigens of streptoc-
coccus 89R50 and of pneumococcal strain R36NC, isolated from cell walls by the trichloro-
acetic acid procedure and purified by diethylami-
noethyl-cellulose chromatography as des-
cribed previously (17). Both the carbohydrate of strain 89R50 and the C polysaccharide of pneumococcus contained glucosamine, galac-
tosamine, glucose, and phosphorus. It is note-
worthy that muramic acid, a structural compo-
nent of mucoprotein, was observed in abun-
dance in the pneumococcal C polysaccharide but was present in only moderate amounts in the cell wall preparation from strain 89R50. The presence of muramic acid in the former clearly indicates that pneumococcal C polysaccharide includes a mucoprotein fraction, and this finding is in essential agreement with that reported previously (11, 23). In addition, it should be noted that the mole ratio of glucosamine to galactosamine in the antigen from streptococ-
coccus 89R50 was markedly different from that of the C polysaccharide of pneumococcus. These findings indicate that, although the cell wall antigens of both the streptococci and pneumo-
coccus possess similar chemical constituents, differences exist which account for their noni-
dentical immunochemical behavior.
Cross-reactivity between the cell wall antigen of streptococcal strain 89R50 and pneumococcal C polysaccharide. Although antibodies to cell wall antigens of streptococcus 89R50 were produced by rabbits immunized with vaccines of this organism, the titer of such antibodies was consistently low. Because of this finding, antiserum to the Cₘ polysaccharide of pneumococcus, rich in cross-reacting antibodies to the cell wall antigen of strain 89R50, was used in this study. Illustrated in Fig. 1 are the results of quantitative precipitin tests performed with pneumococcal C polysaccharide, the cell wall antigen of strain 89R50, and pneumococcal anti-Cₘ serum. Both the streptococcal and pneumococcal cell wall antigens reacted significantly with this antibody. The heterologous reaction between the cell wall antigen of streptococcal strain 89R50 and anti-Cₘ serum was approximately 40% of the homologous reaction between pneumococcal C polysaccharide and anti-Cₘ serum.

Pneumococcal C polysaccharide is composed of two major heteropolymers: teichoic acid and mucopeptide (11, 23). It has been shown that galactosamine-PO₄ is the immunodominant determinant of the teichoic acid (11), whereas the immunochemical determinants of the mucopeptide are diverse. Mucopeptide is composed of three principal moieties: a hexosamine polymer consisting of repeating units of N-acetylglucosamine and N-acetylmuramic acid, a peptide chain linked to muramic acid residues of the hexosamine polymer, and amino acid or peptide bridges which cross-link the peptide chains attached to the hexosamine polymer (27). Although in all instances studied (17-20) these three components of mucopeptide have been found to be antigenic, the peptide consisting of D-alanyl-D-alanine has been shown to be the immunodominant determinant (20, 29). Noteworthy also are the findings of Schifman et al. (28) that pneumococcal C and Cₘ polysaccharides possess a multiplicity of antigenic determinants including the teichoic acid moiety, the mucopeptide moiety, and perhaps the region of attachment of the latter to the teichoic acid moiety. It is possible that any of the components of pneumococcal C polysaccharide, singly or in combination, may function as the antigenic determinant(s) responsible for the cross-reactivity between the cell wall antigens of some filamentous streptococci and pneumococcus.

To elucidate the immunochemical basis for the cross-reactivity between the cell wall antigen of streptococcal strain 89R50 and antibody to pneumococcal Cₘ polysaccharide, the following experiments were designed. Emphasis was placed upon determining the relative immunochemical importance of the teichoic acid and mucopeptide moieties and their relationship to the cross-reaction between the streptococcal cell wall antigen and anti-Cₘ pneumococcal serum. Table 2 is a comparison of the chemical compositions of several purified bacterial mucopeptides. Note that, in all instances, muramic acid, glucosamine, glutamic acid, and alanine were

### Table 1. Chemical composition of cell wall carbohydrates isolated from streptococcal strain 89R50 and pneumococcal strain R36NC

<table>
<thead>
<tr>
<th>Component</th>
<th>Strain 89R50</th>
<th>Strain R36NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucosamine</td>
<td>0.33</td>
<td>0.41</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>0.14</td>
<td>0.34</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.97</td>
<td>0.38</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>2.16</td>
<td>0.99</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.02</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serine</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Muramic acid</td>
<td>0.09</td>
<td>1.04</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>Ammonia</td>
<td>0.12</td>
<td>0.30</td>
</tr>
</tbody>
</table>

* Numbers represent amount of each component, in micromoles per milligram, present in each strain.
the common structural components of mucopeptides derived from both gram-negative and gram-positive bacteria. On the other hand, diaminopimelic acid (DAP) has been shown to be a structural component of the mucopeptides of gram-positive and gram-negative bacilli, whereas lysine has been found in its place in gram-positive cocci. In addition, the peptide bridges of these mucopeptides have been shown to differ in many instances. Figure 2 shows the results of quantitative precipitin reactions between purified mucopeptides rich in lysine and those rich in DAP and a selected antiserum to mucopeptide derived from rabbits immunized with a group A-variant streptococcal vaccine. In all instances, a significant precipitin reaction was observed between each mucopeptide and the selected anti-mucopeptide serum. The results suggest that this antiserum contains antibodies to the hexosamine polymer or to the peptide chains of mucopeptide but little or no antibody to the cross-linking peptide bridges. Quantitative precipitin inhibition tests using various synthetic polypeptides of D-alanine and N-acetylglucosamine clearly indicated that the antibodies present in the selected antimucopeptide serum are directed predominantly against the peptide moiety rather than the hexosamine moiety of the mucopeptides. In addition, the quantitative precipitin inhibition tests showed that the antibodies are directed predominantly against the terminal D-alanyl-D-alanine. This conclusion is based upon data indicating that neither lysine nor DAP plays a role in the mucopeptide precipitin reaction and that D-alanyl-D-alanine has been shown to be a potent inhibitor of that reaction. The results indicate that the selected antimucopeptide serum is rich in antibodies to the C-terminal end of the peptide chains consisting of D-alanyl-D-alanine dipeptides and is virtually devoid of antibodies to the hexosamine moiety of mucopeptide. This monospecific antiserum to mucopeptide was used in immunochemical studies to determine the immunochemical basis for the cross-reactivity of the streptococcal cell wall antigen and an antiserum to pneumococcal C4 polysaccharide.

Table 3 shows the results of chemical analyses of the mucopeptides of streptococcal strain 89R50 and pneumococcal R36NC extracted from cell walls by the hot trichloroacetic acid (18). Note that the compositions of these mucopeptides are similar to those of other bacterial mucopeptides (Table 2) and consistent with findings obtained by other investigators (11, 13, 17, 18, 21, 23, 27). Figure 3 shows the results of immunological studies of the streptococcal and pneumococcal mucopeptides described in Table 3 together with those of the purified mucopeptide of a group B beta-hemolytic streptococcus. Analysis of the quantitative precipitin reactions, depicted in the left frame indicates that the mucopeptides of streptococcal strain 89R50 and of the pneumococcus gave little or no reaction with the selected antiserum to mucopeptide rich in antipeptide antibodies. In contrast,
Table 3. Chemical composition of mucopeptides isolated from streptococcal strain 89R50 and pneumococcus R36NC

<table>
<thead>
<tr>
<th>Component</th>
<th>Streptococcus 89R50</th>
<th>Pneumococcus R36NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucosamine</td>
<td>0.90</td>
<td>0.76</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>0.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Muramic acid</td>
<td>1.00</td>
<td>0.78</td>
</tr>
<tr>
<td>Alanine</td>
<td>1.70</td>
<td>0.77</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.88</td>
<td>0.47</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.80</td>
<td>0.35</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.24</td>
<td>0.16</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.28</td>
<td>0.18</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.13</td>
<td>0.08</td>
</tr>
<tr>
<td>Serine</td>
<td>0.20</td>
<td>0.11</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.10</td>
<td>0.07</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.20</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*Expressed as in Table 1.

Figure 3. Quantitative precipitin reactions between purified streptococcal group B mucopeptide, the mucopeptides of streptococcus type 83 and pneumococcus R36NC, and 0.1 ml of antiserum to the mucopeptide of group A-variant streptococcus (left frame) and 0.1 ml of antiserum to C₈ pneumococcal polysaccharide (right frame).

these same mucopeptides gave moderate reactions with antiserum to C₈ pneumococcal polysaccharide, whereas that of the group B streptococcus gave none. In view of the specificity of the anti-mucopeptide serum, the findings can be interpreted to suggest that the antiserum to pneumococcal C₈ polysaccharide is essentially devoid of antibodies to D-alanyl-D-alanine but may contain antibodies to the hexosamine moiety or to some other component of pneumococcal mucopeptide. Because of the possibility that antibodies to the hexosamine moiety of mucopeptide could be present in the pneumococcal antiserum, it was absorbed with a purified heterologous mucopeptide from a group B streptococcus.

Figure 4 shows the quantitative precipitin reactions between pneumococcal C polysaccharide, the cell wall carbohydrate of streptococcal strain 89R50, both extracted with cold trichloro-acetic acid, and antiserum to pneumococcal C₈ polysaccharide before and after absorption. The amounts of antibody precipitated by the cell wall carbohydrates of both the streptococcus and the pneumococcus from the antiserum both before and after absorption with purified mucopeptide are essentially the same. These findings strongly suggest that the cross-reactivity between the cell wall carbohydrates and the antiserum to pneumococcal C₈ polysaccharide is not related to the mucopeptide moiety of the former. Rather, the observed cross-reactivity between the cell wall carbohydrates of streptococcal strain 89R50 and of pneumococcus may be a reflection of the fact that both polymers include teichoic acid moieties which possess similar immunological determinants.

Enzymatic analysis of the cell wall carbohydrate of streptococcal strain 89R50. Glucose is a major component of the cell wall carbohydrate of streptococcus 89R50, and it is present also in the C polysaccharide of pneumococcus (Table 1). In view of the presence of glucose residues in both of these polymers, the possibility that they may serve as immunodeterminants of their cross-reactivity was investigated. Samples of the cell wall antigen of strain 89R50 were treated with α-D-glucosidase or β-D-glucosidase and subjected to paper chromatography (12, 25). In
this study, only antigen treated with α-D-glucosidase, when chromatographed, revealed free glucose; β-D-glucosidase had no effect upon it. It is noteworthy that the cell wall antigen of streptococcus 89R50 after treatment with α-D-glucosidase still gave a strong precipitin reaction with antiserum to pneumococcal C₆ polysaccharide. This observation is in accord with the results of chemical analysis of the cell wall antigen of strain 89R50 after treatment with α-D-glucosidase. The enzymatically treated antigen was separated into two fractions, one dialyzable, the other not. Analyses of the two fractions are presented in Table 4. Chemical analysis of the enzyme-treated antigen showed a net loss of approximately 50% of its glucose residues, whereas no reduction in the content of hexosamine was observed. The result suggests that the reduction of total glucose in the nondialyzable fraction was due to the release of D-glucose from the polymer. That this is the case is shown in column 3 of Table 4, which indicates that the dialyzable fraction contains significant amounts of free D-glucose residues as determined by the D-glucose oxidase test. The findings indicate clearly that the glucose residues of the cell wall antigen of streptococcal strain 89R50 are not involved in its cross-reactivity with pneumococcal C polysaccharide. This observation notwithstanding, α-D-glucose residues may still be antigenic determinants of the cell wall antigen of strain 89R50 in its reactions with other antisera.

**DISCUSSION**

The alpha- and nonhemolytic streptococci of the human respiratory tract comprise a large and heterogenous group of organisms which produce capsular and cell wall antigens of diverse composition (2). Previous attempts to classify most of these organisms have been largely unsuccessful because of inadequate knowledge of the immunochemical properties of their capsular and cell wall antigens. A recent systematic study of a large number of capsulated filamentous streptococcal strains isolated from human respiratory secretions has resulted in the recognition of more than 30 capsular serotypes (2). In an extension of these observations, it was shown also that many of these strains possess cell wall antigens which cross-react significantly with C polysaccharide of pneumococcus (2). Preliminary evidence has suggested that the cross-reacting cell wall antigens of these filamentous streptococci have similar immunological features and might represent, therefore, a common group-specific antigen. Previous studies have shown that the cell wall antigen of the prototypic strain of filamentous streptococcal capsular type 83 contains galactosamine, glucosamine, glucose, and phosphorus (16). Although an immunological relationship was demonstrated between this antigen and pneumococcal C polysaccharide, the immunochemical basis for the cross-reactivity was not defined.

Most preparations of purified pneumococcal C polysaccharide tend to be quite heterogeneous with respect to their molecular weights, which range from 65,000 to over 200,000 (11). This heterogeneity could account, in part, for differences in the structure of C polysaccharide proposed by several investigators (6, 11, 23). Gotschlich and Liu (11) suggested a structure which consisted of repeating units of β-N-acetyl-D-galactosamine-1-phosphate (teichoic acid) cross-linked to a mucopptide moiety by phosphodiester bonds attached to the muramic acid. Brundish and Baddiley (6) proposed that the teichoic acid of pneumococcus was composed of repeating units of N-acetylglactosaminylglycosyl-ν-acetyldiaminotrideoxyhexosylribitol-phosphate with choline phosphate attached to the ribitol. Mosser and Tomasz (23) suggested a structure for C polysaccharide in which a teichoic acid similar to that proposed by Brundish and Baddiley (6) is attached to the mucopptide as indicated by Gotschlich and Liu. More recently, Bornstein et al. (5) isolated from mutant pneumococci a cell wall-like capsular polysaccharide designated C₆. Immunochemical studies demonstrated that the C₆ capsular antigen was very similar to, though not identical with, the C or cell wall polysaccharide of pneumococcus. Further study of the C and C₆ polysaccharides of pneumococcus by Schiffman et al. (28) showed that subtle immunological differences exist both among the C and C₆ polysaccharides of diverse strains of this organism. By using selected antisera containing high titers of antibody to either the teichoic acid or mucoprotein moiety of C and C₆ polysaccharide,

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**Table 4. Glucose and hexosamine content of the cell wall carbohydrate of streptococcal strain 89R50 before and after hydrolysis with α-D-glucosidase**

<table>
<thead>
<tr>
<th>Streptococcal strain</th>
<th>Glucose content (μg/mg) of carbohydrate from:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-dialyzable</td>
<td>Dialyzable</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>Hexosamine</td>
</tr>
<tr>
<td>89R50</td>
<td>215.5</td>
<td>260.5</td>
</tr>
<tr>
<td>89R50 treated with enzyme</td>
<td>108.6</td>
<td>324.7</td>
</tr>
</tbody>
</table>

* Expressed as in Table 1.
these investigators were able to show that the immunological differences among them resided in the mucopeptide moiety or in the region of its attachment to the teichoic acid. This conclusion is consistent with the observation of Brundish and Baddiley that the teichoic acids of several pneumococcal strains were identical.

The present studies describe the immunological basis for the cross-reactivity between the cell wall antigen of a filamentous streptococcus and antiserum to pneumococcal C₄ polysaccharide. It has been shown that the cell wall antigen of the noncapsulated variant, 89R50, derived from the alpha-hemolytic filamentous streptococcus of provisional capsular type 89, consists of galactosamine, glucosamine, glucose, and phosphorus. It has also been shown that this antigen reacts significantly with antibodies to the heterologous C₄ polysaccharide of pneumococcus. Immunochemical analysis prompted by this observation indicates that antibodies to the teichoic acid moiety rather than to the mucopeptide moiety of the pneumococcal C₄ antigen are responsible for their cross-reactivity with the cell wall antigen of streptococcal strain 89R50. Antiserum to pneumococcal C₄ polysaccharide absorbed with purified group B streptococcal mucopeptide still reacted strongly with the cell wall antigen of strain 89R50.

At present, detailed knowledge of the structure of the cell wall antigen of strain 89R50 is lacking. Partial acid hydrolysis of the antigen has failed to yield any oligosaccharides which could be used in the elucidation of its structure. Studies of enzymatic hydrolysis with α-D-glucosidase, however, have indicated that approximately 50% of the glucose residues of the cell wall polymer of strain 89R50 are alpha-linked terminal residues of D-glucose. Although 50% of the glucose residues were removed from the antigen by the enzyme, it still reacted strongly with antiserum to pneumococcal C₄ polysaccharide. It is evident that the terminal alpha-linked glucose residues do not play a significant role in the cross-reactivity of the streptococcal cell wall antigen with antibody to pneumococcal C₄ polysaccharide. These residues may be nonetheless important immunological determinants of the cell wall antigen of this, and perhaps other, filamentous streptococci. This question can only be resolved when suitable antibody to the streptococcal cell wall antigen becomes available and attempts to obtain it continue. The availability of such a reagent could aid appreciably attempts to develop an immunological scheme for the classification of the alpha- and nonhemolytic streptococci of the human respiratory tract.

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LITERATURE CITED


